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$AlCl_3$ causes Fas/Fas-L mediated cell death in the cortex and hippocampus of mouse brain

Rohini Keshava[1], Vikas Vazhayil[2], Rohan Mitra[3], Indira Devi Bhagavatula[4] and Rajalakshmi Gope[5]
1 Department of Biotechnology, Faculty of Life and Allied Health Sciences, Bangalore 560054, India.
2,4 Department of Neurosurgery, NIMHANS, Bangalore 560029, India.
3 Medical Writer, Sanofi, Mumbai 400072, India.
5 Department of Human Genetics, NIMHANS, Bangalore 560029, India.
rohinikeshavaofficial@gmail.com; vikas.drv@gmail.com; mitrohan@gmail.com; bidevidr@gmail.com; rlgope@gmail.com

ABSTRACT
In this study effect of sustained toxicity of low dose of aluminum trichloride ($AlCl_3$, Anhydrous) in mouse brain and liver was evaluated. Six weeks old Swiss albino mice were given orally 2 mg $AlCl_3$/ kg body weight / per day for three consecutive weeks. Three weeks after last feeding the brain and liver tissues were examined for histopathological changes which revealed Al toxicity related changes. Oral administration $AlCl_3$ caused a slight decrease in the whole-body weight and a significant decrease in the weight of liver and brain tissues. In addition, $AlCl_3$ ingestion resulted in increased level of total protein in the liver but decreased the same in the brain tissue in the experimental mice. $AlCl_3$ feeding also caused DNA laddering that is characteristics of apoptotic cell death in the liver and brain tissues. Moreover, $AlCl_3$ feeding lead to decreased level of anti-apoptotic Bcl2 and PI3K proteins, and increased levels of p53 as well as pro-apoptotic phospho c-Jun N-terminal kinase (pJNK), Bax, Caspase 3, p21 and Fas proteins in the cortex and in the hippocampus regions. Trace level of Fas protein was present in the cortex and hippocampus regions of the control brain which increased to 2 to 3-fold upon $AlCl_3$ feeding. Higher level of Fas ligand was present in the cortex and hippocampus regions of the control and it remained unchanged with $AlCl_3$ ingestion. Our data shows that the observed cell death caused by $AlCl_3$ in the cortex and hippocampus region of mouse brain could be Fas mediated.

Running title: Aluminium chloride causes Fas mediated cell death
Key words: $AlCl_3$, apoptosis, Bcl2: Bax ratio, cell death/survival, Fas/Fas-L, p53

INTRODUCTION
Aluminium chloride ($AlCl_3$) causes neuronal degeneration in animal models and accumulation of aluminum (Al) produces neurodegenerative disorders in human [1,2]. Several independent studies have demonstrated that Al accumulation causes liver toxicity [3,4] as well as neuronal cell death [5]. Al ingestion causes histopathological changes in the liver and brain tissues in animal models and in humans [4-6]. $AlCl_3$ toxicity causes accumulation of p53 protein in the brain cells [6]. Al is suggested to induce apoptotic cell death in the neurons in p53 dependent and p53 independent manner [6,7]. Dysregulation of a variety of proteins including the cell survival proteins Bcl2 [8] and PI3K [9] and cell death proteins such as phospho JNK [10], Bax [11], Caspase 3 [12], p21 [13], Fas [14] and Fas ligand [15] are involved in apoptotic cell death pathways. In the present study the effect of daily oral administration of low dose of 2 mg/ kg /per day of $AlCl_3$ was evaluated in mice model. Effect of $AlCl_3$ on the histopathological changes in the liver and brain cells were evaluated by microscopic examination of the paraffin embedded tissue sections stained with haematoxylin and eosin. We have also examined the effect of $AlCl_3$ on the DNA integrity in the liver and brain tissues. In addition, the levels of p53 protein and many other cell survival and cell death proteins that are known to be involved in apoptotic pathway were analysed in the $AlCl_3$ fed mice as compared to the control. Our data confirmed that the daily oral administration of low dose of $AlCl_3$ caused characteristic histopathological changes associated with Al toxicity, produced DNA fragmentation and induced apoptotic cell death in the cortex and hippocampus regions. We found modulation of expression of the various cell survival and

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cell death proteins in the $AlCl_3$ fed mouse brain as compared to the control. Notably, the level of Fas protein increased significantly in the cortex and hippocampus region upon $AlCl_3$ feeding. Our results indicate that the observed cell death in the cortex and hippocampus regions could be through Fas mediated apoptotic pathway.

MATERIALS AND METHODS

1. Animals

This study was conducted after obtaining the approval from the institutional Animal Ethics Committee, No. AEC/58/360/HG. Four-week-old mice (Swiss Albino) were received from the Central Animal Research Facility (CARF), NIMHANS and kept at room temperature (30° C) in polypropylene cages with a relative humidity of approximately 50% and light-dark cycle of 12 hours. The mice were kept under these conditions for two additional weeks to acclimatise in the experimental room. During this period and throughout the experimental period the mice were fed water *ad libitum* and commercially available pelleted mouse feed [16].

2. Oral administration of $AlCl_3$

The six-week-old Swiss albino mice weighed approximately 30 g each. These mice were fed orally 2 mg/ kg body weight / day $AlCl_3$ (anhydrous, Loba Chemie, Wode House Road, Colaba, Mumbai 400 005, India) dissolved in tap water for three consecutive weeks. A stock solution of six milligram per ml of $AlCl_3$ was prepared in tap water. Ten microliters of this stock solution containing 60 micrograms of $AlCl_3$ was diluted in a total volume of 500 microliter of tap water and immediately fed once a day to the mice orally using a 1 ml micropipette tip for three consecutive weeks. At the end of the $AlCl_3$ feeding the mice were 9 weeks old. These mice were kept without any $AlCl_3$ feeding for 3 additional weeks. Then the 12-week-old mice were used for further analysis. Four mice each were used as control and for $AlCl_3$ feeding and the experiments were repeated thrice.

3. Monitoring of body weight, total protein in liver and brain

The weight of the control and $AlCl_3$ fed mice were monitored daily. The whole liver and brain were collected, washed thrice in phosphate buffered saline (PBS), blotted off the excess PBS with tissue paper and weighed individually. The whole-body weight and the wet weights of whole liver and brain were obtained individually for 12 mice. To make a functional comparison the individual liver and brain weights were adjusted to per 100 g of body weight and expressed as percentage change. The total protein from the whole liver and brain tissues were isolated using Tri-reagent (Sigma-Aldrich, St Louis, USA) and their concentrations were measured using spectrophotometer. The quantity of total proteins from liver and brain were normalised to the weight of whole liver and brain and expressed as percentage change. The experiment was repeated thrice to obtain statistically significant data.

4.Tissue collection and histopathological studies

Three weeks after the last feeding the mice were 12 weeks old. The control and $AlCl_3$ mice were sacrificed by cervical dislocation and the brain and liver tissues were collected, washed three times in phosphate buffered saline (PBS) and one half of brain and liver from all animals was fixed in 10% formalin in PBS and the other half was immediately frozen in -80° C until use. Structural changes in the brain and liver were analysed using 10-20 µm thickness paraffin sections stained with haematoxylin and eosin followed by examination under light microscope (BX-51, Olympus, Singapore). The histopathological changes in the liver were also used as an indicator for the cytotoxicity induced by $AlCl_3$ [4].

5. DNA laddering in mouse brain

The DNA laddering in the brain and liver tissues was used as a marker for apoptotic cell death. The DNAs were isolated from the liver tissues as well as from the cortex and hippocampus regions of $AlCl_3$ fed and control

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mice using Tri-reagent (Sigma-Aldrcih, St Louis, USA) according to the protocol supplied by the manufacturer. Two to 3 µg of DNA samples were separated on a 1.2 % agarose gel containing 0.001% ethidium bromide, then visualised the DNA using a gel doc system and photographed (Syngene, Cambridge, UK). The phage lambda DNA digested with Hind III (Sigma-Aldrich, St Louis, USA) was used as a molecular weight standard [17].

6. Preparation of brain tissue homogenate

From a series of 40 µ thick sagittal sections of the whole brain the cortex and the hippocampus regions were dissected under a 20x magnifying lens. The dissected cortex tissues comprised of more than 95% of cortex cells and the hippocampus tissue comprised of more than 90% of hippocampus plus 10% of dentate gyrus cells. These tissues were individually suspended in cold lysis buffer at the ratio of 100 µL of buffer for each 10 mg wet weight of tissues and homogenised using a Polytron (Kinematica, Switzerland) for 30 seconds x 5 with 30 seconds cooling on ice in between strokes. The cell debris were removed by centrifugation at 10,000 rpm for 10 minutes at 4° C, the clear lysates were transferred to fresh tubes and used for Western blot analysis [17].

7. Western blot analysis

One hundred fifty micrograms of total protein from each tissue lysates were separated on 7.5% SDS-PAGE gels, transferred to PVDF membrane (Sigma-Aldrich, St Louis, MO, USA) using a semi-dry blotter (Bio-Rad, Australia). The membranes were analysed for the levels of expression of p53, Bcl2, PI3K, pJNK, Bax, Caspase 3, p21, Fas and Fas-L proteins using appropriate primary and corresponding secondary antibodies and the level of γ-tubulin protein was used as a control. The pre-stained broad range, high molecular weight marker (sc-2361, Santa Cruz Biotechnology, Santa Cruz, CA, USA) was used as a standard to determine sizes of the protein bands. The primary and secondary antibodies were obtained from Santa Cruz Biotechnology, Santa Cruz, CA, USA, unless and otherwise mentioned. The experiments were run in duplicate sets. The membranes from one set were probed individually with the primary antibodies for p53 (sc-126, 1:500), Bcl-2 (sc-7382, 1:1000), PI3K (#SAB2500792, 1:1000), JNK (J4750, 1:1000), Bax (sc-7480, 1:1000), Caspase 3 (C9598; 1:1000), p21 (#SAB4300419, 1:1000,), Fas (sc-715, 1:1000) and Fas-L (sc-834, 1:1000); the corresponding second set of membranes were probed with γ- tubulin (clone GTU-88, T5326; 1:1000) antibody for 2 hours. The PI3K, JNK, Caspase 3, p21 and γ-tubulin antibodies were obtained from Sigma-Aldrich, St Louis, MO, USA.

The membranes were washed thrice, further incubated with corresponding, AP conjugated secondary antibodies for 1 hr, again washed thrice and developed with one step NBT-BCIP (Sigma-Aldrich, St Louis, MO USA) as described previously [17]. The levels of various proteins were first normalised to the γ-tubulin protein levels in the same lane which was used as an internal control in the Western blots. After such normalisation the levels of p53, Bcl2, PI3K, pJNK, Bax, Caspase 3, p21, Fas and Fas-L proteins were compared between the control and $AlCl_3$ fed mice samples.

8. Statistical analysis of data

The results on the changes in weights of whole animal, liver and brain were calculated expressed as mean ± standard deviation (SD). The levels of the various proteins were first normalised to the γ-tubulin protein levels in the same lane which was used as an internal control in the Western blots. After normalisation the level of p53, Bcl2, PI3K, JNK, Bax, Caspase 3 p21, Fas and Fas-L proteins were compared between the control and $AlCl_3$ fed mouse brain samples. Statistical Package for Social Sciences (SPSS) version 16.0 was used to analyse data obtained from the $AlCl_3$ fed mice and compared to that of the control. Paired t-test and repeated measures analysis of variance (RMANOVA) were carried out for the values obtained from all the individual

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samples (N=12). The p <0.05 was considered as statistically significant [17].

RESULTS

1. Effect of $AlCl_3$ on the body, liver and brain weights and the level of total protein

The $AlCl_3$ fed mice weighed approximately 3 to 5% less than that of the control. The average wet weight of whole liver in $AlCl_3$ mice was approximately 20% less than that of the control (Figure 1). However, the level of total protein in the $AlCl_3$ liver increased by 23% and these data were statistically significant with a $p < 0.05$ (Figure 2). The average wet weight of whole brain in the $AlCl_3$ fed mice was approximately 8% less as compared to the control (Figure 1). In addition, the level of total protein in the $AlCl_3$ brain decreased by 12% as compared to the control and these data were statistically significant with a $p < 0.05$ (Figure 2).

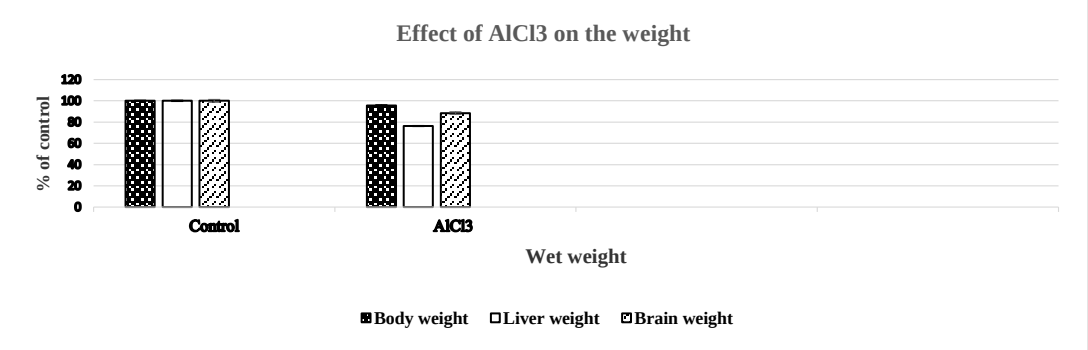

Figure 1: Effect of $AlCl_3$ on the body, liver and brain weights. The values represent means ± SD. n = 12 for each group.

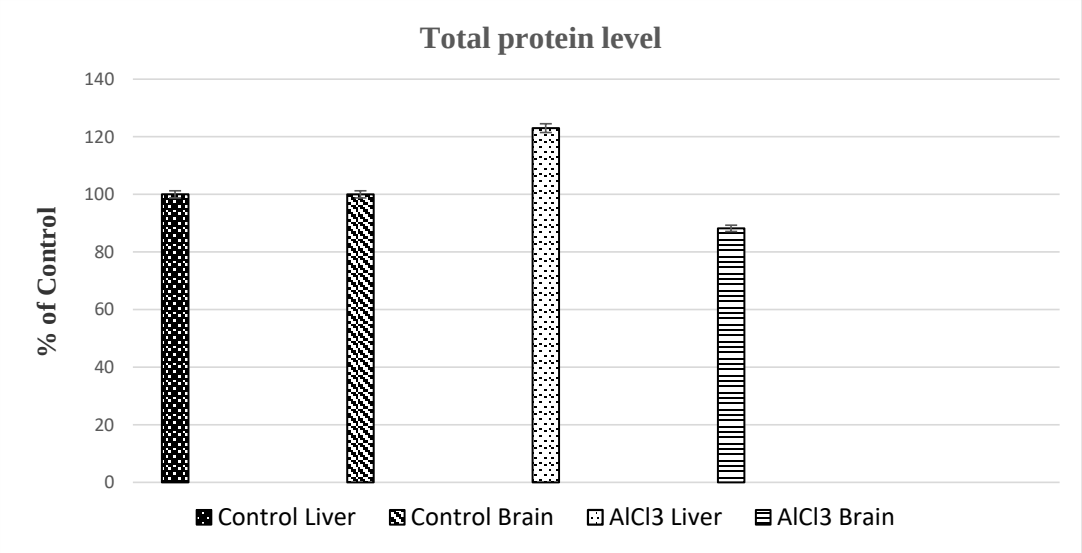

Figure 2. Effect of $AlCl_3$ on the level of total protein. The control indicates the total protein level in the liver and in brain which was taken as 100%. The values are given as means ± SD. n = 12 for each group.

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2. Effect of $AlCl_3$ on the histopathology of liver and brain

The histopathology of the liver from control mice showed normal liver histology and cell structure. The control liver tissue showed normal arrangement of hepatocytes, a few binucleated cells and normal sized sinusoids. However, the histopathology of $AlCl_3$ fed liver showed hepatic injury marked by medium level of hepatocellular necrosis distributed randomly throughout the parenchyma. In addition, $AlCl_3$ liver also showed increased inflammatory cell infiltration, vascular congestion, dilated sinusoid and moderate vacuolation in the cytoplasm and pyknotic nuclei indicative of Al induced toxicity (Figure 3).

The histology of control brain sections showed a normal cellular architecture in the cortex and hippocampus regions. The sparse necrotic regions were found in the cerebral cortex of the $AlCl_3$ fed mice as compared to the control (Figure 4a). $AlCl_3$ feeding also caused cell death in the CA1 and dentate gyrus (DG) regions (Figure 4b). The histopathological changes in the liver and brain tissues were consistent in all three experiments.

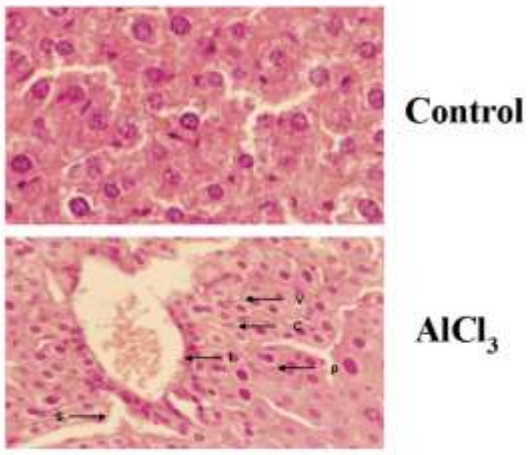

Figure 3

Figure 3: Histopathological effect of $AlCl_3$ on the liver. The control and $AlCl_3$ fed liver histopathology are as indicated. The top panel shows control with normal liver architecture. The bottom panel shows post $AlCl_3$ treatment. The arrows indicate changes caused by $AlCl_3$ toxicity. c, cellular condensation; i, cellular infiltration; p, karyopycnosis; s, enlarged sinusoid; v, vacuole. Magnification 100 x.

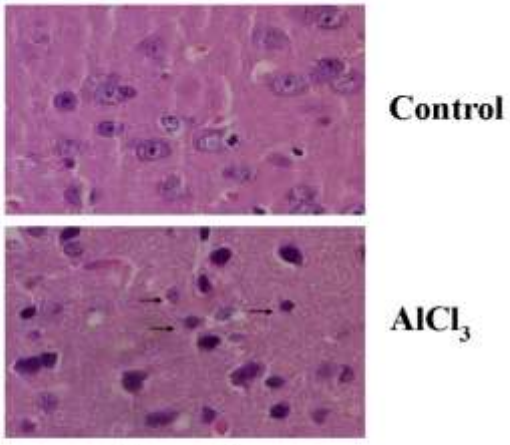

Figure 4a

Figure 4a: Histopathological effect of $AlCl_3$ on the cortex region of moue brain. The top panel shows the control cortex and the bottom panel shows the $AlCl_3$ fed cortex. Some of the dead cells in the cortex are shown by arrows. Magnification 100 x.

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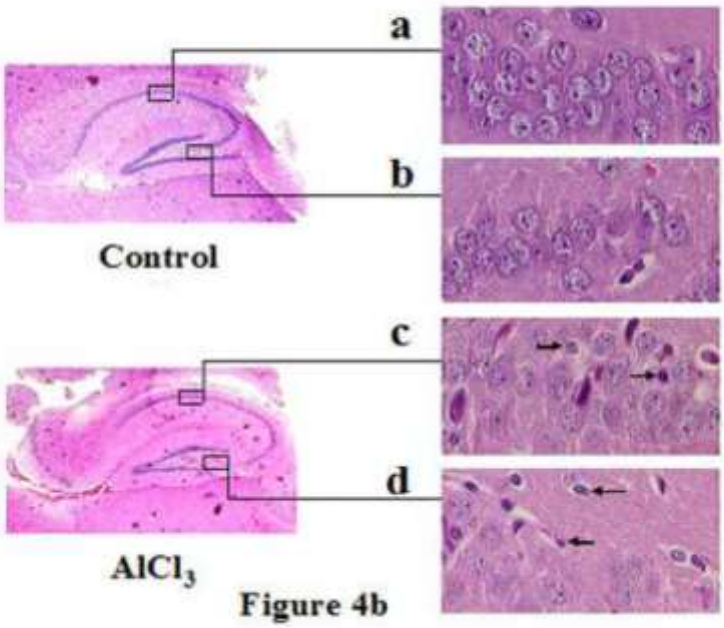

Figure 4b

Figure 4b: Histopathological effect of $AlCl_3$ on the hippocampus region of mouse brain. Low magnification of the control (top, left) and $AlCl_3$ fed (bottom, left) sagittal section of the mouse brain is as shown. Magnification 20 x.

The corresponding higher magnification of the CA1 (a) and dentate gyrus (DA) (b) regions are shown on the right-side panel. The dead cells in the CA1 (c) and DA (d) regions in the $AlCl_3$ fed mice are shown by arrows. Magnification 100 x.

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3. Effect of $AlCl_3$ on the DNA integrity

The control brain and liver samples showed no DNA laddering. However, the DNA from the brain and liver tissues showed characteristic DNA laddering associated with apoptotic cell death. The DNA laddering was found in both the cortex and hippocampus regions of the $AlCl_3$ fed mice brain (Figure 5).

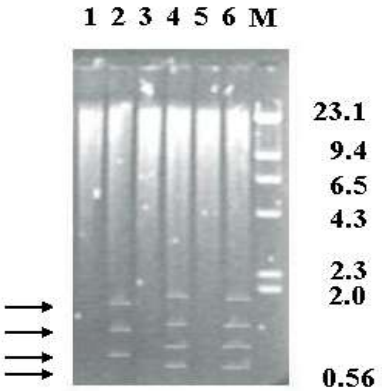

Figure 5

Figure 5: DNA laddering caused by $AlCl_3$. Lane 1, Control liver, Lane 2, $AlCl_3$ liver; Lane 3, Control cortex, Lane 4, $AlCl_3$ cortex, Lane 5, Control hippocampus, Lane 6, $AlCl_3$ hippocampus, M, DNA molecular weight marker, bacteriophage lambda DNA digested with Hind III; the standard molecular weights are as indicated on the right. The arrows on the left indicated the various size of bands in the DNA ladder.

4. Effect of $AlCl_3$ on the levels of p53

Minimum level of p53 protein was found in the cortex and hippocampus regions of control mouse brain. Oral administration of $AlCl_3$ caused 1.5- and 2.2-fold increase in the level of p53 protein in the cortex and hippocampus regions respectively (Figure 6a). The results were significant with a $p < 0.05$.

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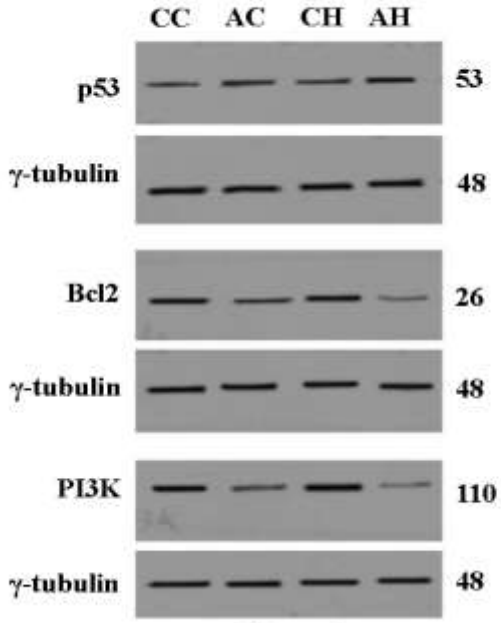

Figure 6a

Figure 6a: Effect of $AlCl_3$ on the levels of p53, Bcl2 and PI3K proteins. The various samples used are given on the top of the figure. CC, control cortex; AC, $AlCl_3$ cortex; CH, control hippocampus; AH, $AlCl_3$ hippocampus; the various proteins analysed and the corresponding control γ- tubulin are as indicated on the left. The corresponding molecular weights of the various proteins are given on the right.

5. Effect of $AlCl_3$ on the levels of cell survival proteins Bcl2 and PI3K

A moderate level of cell survival proteins Bcl2 and PI3K were found in the control and $AlCl_3$ feeding caused a 1.2- and 2.0-fold decrease in these proteins respectively in the cortex region. In the hippocampus region the Bcl2 and PI3K proteins decreased further by 1.5 and 2.5-fold respectively (Figure 6a). These results were significant with a $p < 0.05$. Upon $AlCl_3$ feeding the Bax:Bcl2 ratio changed from approximately 1:1.5 to 2.2:1.0 (± 0.001) in the brain (Figure 6a; Figure 6b).

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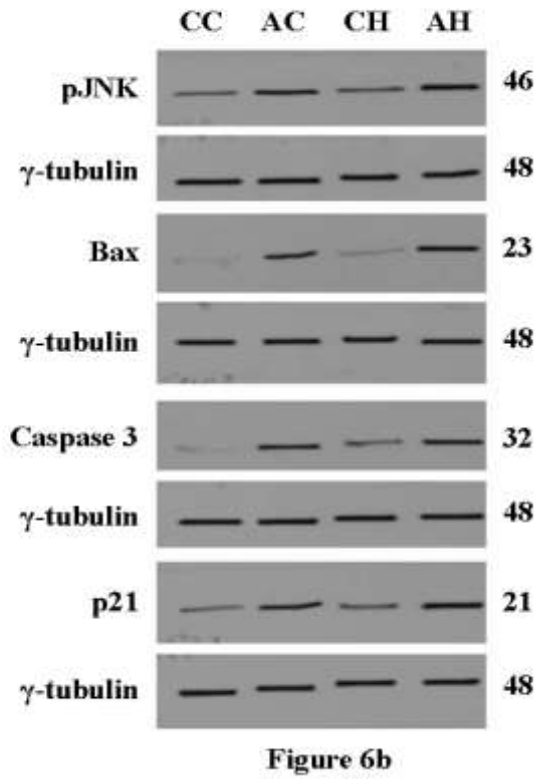

Figure 6b

Figure 6b: Effect of $AlCl_3$ on the levels of pJNK, Bax, Caspase 3 and p21 proteins. CC, control cortex; AC, $AlCl_3$ cortex; CH, control hippocampus; AH, $AlCl_3$ hippocampus; the various proteins analysed and the corresponding control γ- tubulin are as indicated on the left. The corresponding molecular weights of the various proteins are given on the right.

6. Effect of $AlCl_3$ on the levels of cell death proteins pJNK, Bax, Caspase 3, p21, Fas and Fas-L

The JNK antibody recognised only the 46 kDa phosphorylated form (pJNK) and not the 54 kDa under-phosphorylated form. Minimal levels of pJNK and p21 proteins were present in the controls. The level of pJNK increased by 1.5 and 2.0-fold in the cortex and hippocampus regions respectively in the $AlCl_3$ fed mice. The level of p21 increased by 2.2-fold in the cortex and 2.4-fold in the hippocampus of the $AlCl_3$ fed mouse brain (Figure 6b). Trace levels of Bax, Caspase 3 and Fas proteins were present in the control brain. A 2.0-fold increase in Bax protein were found in the cortex region of the $AlCl_3$ fed mice and in the hippocampus region it further increased to a 2.3-fold. Upon $AlCl_3$ feeding the level of Caspase 3 increased by 2.2 and 2.4-fold in the cortex and hippocampus regions respectively (Figure 6b). The level of Fas protein in the $AlCl_3$ fed mice increased by 2.3 in the cortex and 3.0-fold in the hippocampus. These results were significant with a $p < 0.05$.

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A higher level of Fas-L was present in both the cortex and hippocampus regions in the controls and $AlCl_3$ feeding caused little change in these levels (Figure 6c).

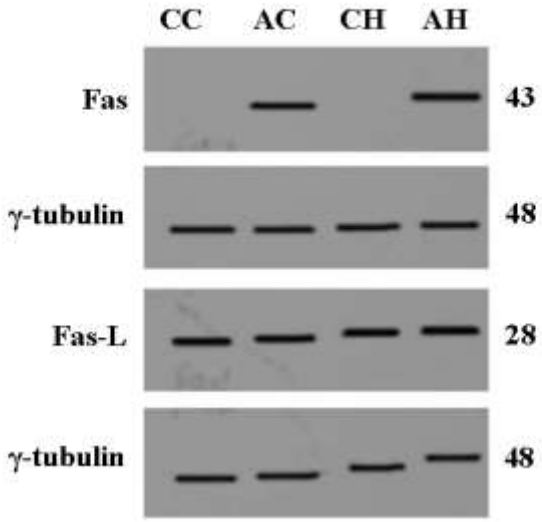

Figure 6c

Figure 6c: Effect of $AlCl_3$ on the levels of Fas and Fas-L proteins. CC, control cortex; AC, $AlCl_3$ cortex; CH, control hippocampus; AH, $AlCl_3$ hippocampus; the various proteins analysed and the corresponding control γ- tubulin are as indicated on the left. The corresponding molecular weights of the various proteins are given on the right.

DISCUSSION

The hepatic weight loss has been reported to be an indicator of Al toxicity [4,18]. A positive correlation between the reduced organ mass and cellular aging and senescence has been reported previously [19,20]. The present data on the reduced liver weight in the $AlCl_3$ fed mice as compared to the control are in agreement with these previously published reports on weight reduction caused by Al in animal model [18] and consequent hepatocyte toxicity and senescence [19,20] (Figure 1). Toxic metals including Al affect synthesis and intracellular processing of proteins [21]. Systemic Al toxicity is comparable to advanced age [19]. Aged liver may synthesise more proteins as a mean to compensate for increased proteinuria, increased proteolysis and accumulation of defective proteins [22] and data from the present study on the

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increased protein level in the liver from $AlCl_3$ fed mice corroborates these findings (Figure 2).

Al ingestion causes its accumulation in the liver where it manifests cytotoxic effects in a dose dependent manner [223,24]. $AlCl_3$ causes hepatocellular necrosis, increased inflammatory cell infiltration, dilated sinusoid, vacuolation in the cytoplasm and pyknotic nuclei in rat liver [4] and our data showed similar histopathological changes in the liver tissue of $AlCl_3$ fed mice (Figure 3).

Other researchers have used many folds higher concentration of Al [19,21,23,24] as compared to the 2 mg / kg body weight / day used in our study. However, we found similar effect on overall body, liver and brain weights and protein levels as well as histopathological changes which is indicative of the persistent potency of even lower dose of Al on a whole host of animal physiology (Figure 1 to 4). In addition, we evaluated the effect of $AlCl_3$ three weeks after cessation of oral $AlCl_3$ feeding. Whereas the other groups have evaluated the effect of Al immediately after the course of administration [19,21,23,24]. In our study the $AlCl_3$ was administered orally as oppose to intraperitoneal injection by another group [25].

Al toxicity causes apoptotic cell death in brain cells as well as in cultured neuronal cells *in vitro* [9,10,26]. Our data on the DNA laddering in the liver, cortex and hippocampus is indicative of $AlCl_3$ induced toxicity followed by apoptotic cell death in these regions (Figure 5).

The p53 gene regulates multiple cellular pathways in various cell types and is also activated in response to stress [26]. Upon stress the p53 protein causes cell cycle arrest, cellular senescence, autophagic cell death and apoptosis [12,27]. A 2-fold increase in the level of p53 transcript was reported in mouse neuroblastoma cell line, Neuro 2a, exposed to aluminium maltolate [26]. We found increased level of p53 protein in the cortex and hippocampus of $AlCl_3$ fed mice as compared to the control (Figure 6a) which relates to Al toxicity. p53 is a transcriptional activator of Bax gene [11]. p53 suppresses pro apoptotic Bax and induces anti-apoptotic Bcl2 *in vitro* and *in vivo* [8]. Our data on the increased ratio of Bax:Bcl2 in $AlCl_3$ fed mouse brain is indicative of pro-apoptotic condition caused by $AlCl_3$ (Figure 6a; Figure 6b).

PI3K participates in multiple cellular processes including cell growth and survival and protection from apoptosis [9]. Constitutively active PI3K delays p53 mediated apoptosis which provides a direct link between p53 mediated apoptosis and PI3K signalling pathway [28]. The present data on the increased level of p53 and reduced level of PI3K in $AlCl_3$ fed mouse brain indicates that the Al toxicity could render neuronal cells more susceptible to apoptotic cell death (Figure 6a).

Al induces apoptotic cell death in the cortical neurons [10,29] and the observed increase in the level of pJNK in the $AlCl_3$ brain together with DNA laddering is indicative of apoptotic cell death (Figure 5; Figure 6b). Increased level of Caspase 3 expedites apoptotic cell death [29] and the observed upregulation of Caspase 3 in this study is in agreement with these reports (Figure 6b). Cell cycle inhibition by p21 is mediated through p53 dependent gene expression that is essential for apoptosis [13,30]. Our data on the increased level of p21 in the $AlCl_3$ fed mice confirms that the DNA fragmentation observed in the cortex and hippocampus is due to apoptotic cell death (Figure 5; Figure 6b).

Reduced level of Bcl2 concurred simultaneous increase in the level of Fas in the hippocampus region of the $AlCl_3$ administered weaning Wistar rats [31]. In the $AlCl_3$ fed mice we found down regulation of Bcl2 accompanied by upregulation of Fas in the cortex and hippocampus regions (Figure 6a; Figure 6c). The controls had trace level of Fas protein. Previously it has been shown that the intraperitoneal administration of higher concentration of 10 mg/ kg $AlCl_3$ for 90 days

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had no effect on the level of Fas and Fas-L in the hippocampus in mice model [25]. However, we found presence of constitutively higher levels of Fas-L in the cortex and hippocampus in the control mice and it remained almost unaffected upon $AlCl_3$ ingestion (Figure 6c). In addition, we also found that the level of Fas protein increased by 2.2- and 2.5-fold increase in the cortex and hippocampus respectively upon oral administration of low dose of 2 mg/ kg body weight of $AlCl_3$ followed by no Al ingestion for 3 weeks (Figure 6c). Our data clearly shows that the $AlCl_3$ toxicity caused more pronounced increase in cell death proteins and concomitant decrease in the cell survival proteins in the hippocampus as compared to the cortex (Figure 6a to 6c).

Some reports have shown that the $AlCl_3$ caused neuronal apoptosis *via* mitochondria-mediated intrinsic pathway [25] and the others have shown that the Al induced cell death *via* p53 mediated extrinsic pathway [26,32]. These discrepancies appear to be due to a variety of factors such as the concentrations of Al used (500 mg, 10 mg, 2mg), the form of Al (Al-maltolate, $AlCl_3$), the mode and duration of Al administration (oral, intraperitoneal) or whether Al was given alone or in combination with other substance (D-galactose), or if the work was done using cultured cells or animal model [25,26,32,33]. The importance of aluminum and its association with certain neurological disorders have been elucidated in a long term, multicentre study [34]. Our data emphasises the importance of ingestion of even lower dose of $AlCl_3$ of 2 mg/kg/day for a short duration of 21 days that causes liver toxicity and neuronal cell death and it appears to be Fas mediated. It is also important to note that we measured the toxic parameters that were persistent three weeks after the last feeding of $AlCl_3$ which implies the long-lasting effect of lower dose of Al-induced toxicity. It is also important to note that there are overlaps between the p53 and Fas- mediated cell death pathways as they have many common factors such as Caspase 3, Bcl2, Bax etc. playing central roles. In the light of our current data any future drug development against Al induced neurodegenerative disorders in human beings perhaps should first establish the possible neuronal cell death pathway which could be either p53-dependent or p53-independent or Fas-mediated. It appears that the various cell death pathways induced by Al could be dependent on its concentration (ranging from 10 to 500 mg /kg/ per day), type of Al ion ($AlCl_3$ Al-maltolate), entry route (oral or intraperitoneal) or its combination with other factors (Al plus D-galactose) or the experimental conditions (cell line, rat, mouse). Our data clearly shows that intake of lower dose of $AlCl_3$ through drinking water source even for a short duration of 3 weeks could trigger a cascade of Fas/Fas-L mediated cell death pathway that is persistent 3 weeks post cessation of further intake of $AlCl_3$. Therefore, any future drug development for neurodegenerative disorders caused by low dose of Al toxicity should focus on disruption of Fas/Fas-L mediated neuronal cell death pathway.

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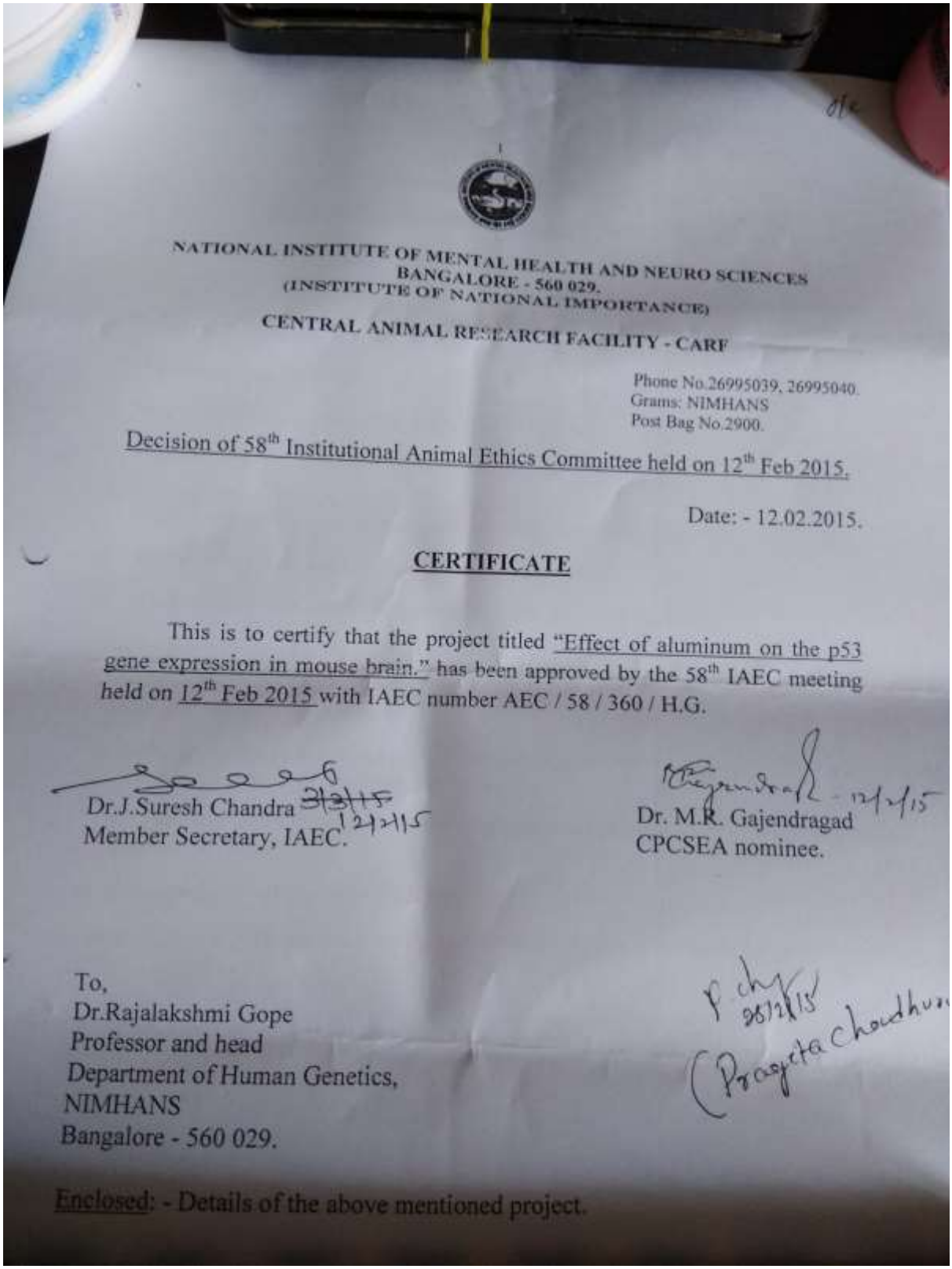

NATIONAL INSTITUTE OF MENTAL HEALTH AND NEURO SCIENCES
BANGALORE - 560 029.
(INSTITUTE OF NATIONAL IMPORTANCE)

CENTRAL ANIMAL RESEARCH FACILITY - CARF

Phone No.26995039, 26995040.
Grams: NIMHANS
Post Bag No.2900.

Decision of 58th Institutional Animal Ethics Committee held on 12th Feb 2015.

Date: - 12.02.2015.

CERTIFICATE

This is to certify that the project titled "Effect of aluminum on the p53 gene expression in mouse brain." has been approved by the 58th IAEC meeting held on 12th Feb 2015 with IAEC number AEC / 58 / 360 / H.G.

Dr.J.Suresh Chandra 12/2/15
Member Secretary, IAEC.

Dr. M.R. Gajendragad 12/2/15
CPCSEA nominee.

To,
Dr.Rajalakshmi Gope
Professor and head
Department of Human Genetics,
NIMHANS
Bangalore - 560 029.

28/2/15
(Pragyta Chaudhu

Enclosed: - Details of the above mentioned project.